NAFCILLIN-INDUCED PLATELET DYSFUNCTION

by

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University of Utah in partial fulfillment of the requirements
for the degree of

Doctor of Pharmacy

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UNIVERSITY OF UTAH COLLEGE OF PHARMACY

FINAL READING APPROVAL

TO THE DOCTOR OF PHARMACY COMMITTEE OF THE UNIVERSITY OF UTAH COLLEGE OF PHARMACY:

I have read the clinical research project report of Donald Page Alexander, Jr. in its final form and have found that 1) its format, citations, and bibliographic style are consistent and acceptable; 2) its illustrative materials including figures, tables, and charts are in place; and 3) the final manuscript is satisfactory to the Supervisory Committee and is ready for submission to the Doctor of Pharmacy Committee.

27 May 1981
Date

Chairman, Supervisory Committee

Approved for the Department of Pharmacy Practice

Chairman

Approved for the Doctor of Pharmacy Committee

Chairman, Doctor of Pharmacy Committee

UNIVERSITY OF UTAH COLLEGE OF PHARMACY

SUPERVISORY COMMITTEE APPROVAL

of a clinical research project report submitted by

Donald Page Alexander, Jr.

We, the undersigned, have read this clinical research project report and have found it to be of satisfactory quality for a Doctor of Pharmacy Degree.

27 May 1981
Date

Chairman, Supervisory Committee

27 May 1981
Date

Member, Supervisory Committee

27 May 1981
Date

Member, Supervisory Committee

DEDICATION

I would like to dedicate this manuscript to my parents, whose understanding and support has given me the strength to fulfill this goal.

I would also like to dedicate this manuscript to the faculty of the Department of Pharmacy Practice, College of Pharmacy, University of Utah. Their knowledge, guidance, dedication, motivation, and enthusiasm have contributed so much to my career.

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BACKGROUND

Many drugs interfere with the normal hemostatic function of platelets.[1,2] A list of drugs which interfere with hemostasis is found in Appendix I. The largest number of reported cases of antibiotic-induced hemostatic defects is associated with carbenicillin,[3-22], and penicillin G.[20-30] These hemostatic defects are identified primarily as platelet dysfunction with some cases resulting in clinical bleeding. Ampicillin,[20,29,30] methicillin,[30] oxacillin,[29] cloxacillin,[17,31,32] ticarcillin,[17,31,32] cephalothin,[20,28,29,33] and cefazolin[34] show similar effects when these drugs are given to animals or humans prior to blood sampling for in vitro aggregation studies. This same dysfunction is thought to occur when normal platelet-rich plasma (PRP) is spiked with these drugs prior to aggregation studies. The exact mechanism of the effect on platelets is unknown.

When a vessel is damaged, platelets adhere to the exposed elements of the subendothelium, i.e., collagen, microfibrils and basement membrane.[35,36] The hemostatic mechanism is initiated with platelet adhesion to the site of injury. After adhesion, aggregation of platelets to each other enlarges the hemostatic plug. Aggregation at the site of injury is facilitated by a number of stimuli. Adenosine diphosphate (ADP), released from damaged tissue, and collagen, released from exposed subendothelium, stimulate platelet aggregation. Epinephrine is present from catecholamine release during the vascular phase of injury and thrombin formation is present from activated coagulation proteins which contribute to the further activation of platelets. Primary aggregation results from direct stimulation of the platelet by the various stimuli. It is considered to be a reversible process. Platelets which aggregate

may begin to disaggregate and recirculate throughout the rest of the body. Secondary aggregation, which follows primary aggregation, is associated with secretion of subcellular constituents[36-39] and stimulation of prostaglandin synthesis.[40] Released ADP and prostaglandins further stimulate platelet aggregation and secretion.[41,42] This process results in an irreversible aggregation of platelets.

The activation of the extrinsic and intrinsic coagulation pathways results in activation of thrombin and the formation of fibrin.[36] The fibrin formation within the platelet mass appears to stabilize the mass as a firm structure. At this point, no one mechanism is predominant; however, they all work together to form a stable clot.

Platelet aggregation studies are used to identify platelet defects. When a proper physiologic stimulus (ADP, collagen, epinephrine) is added to PRP the aggregation response can be quantitated by the change in the optical density as the platelets aggregate. Not all laboratory identified platelet dysfunction results in clinical bleeding nor is there any in vitro test which correlates with the presence of clinical bleeding. Screening individuals with a bleeding time is presently the easiest way to detect altered platelet function, although this is not 100% predictive.[43] Other patient factors such as individual sensitivities, underlying disease, trauma or surgery may predispose the patient to situations where altered platelet function may cause bleeding.

The defect in platelet aggregation induced by antibiotics is more commonly seen with the use of ADP as the aggregating agent. Inhibition of the secondary wave of ADP-induced aggregation is initially observed. At higher concentrations of antibiotics, depression of the primary aggregation response to ADP accompanies the loss of secondary aggregation.[18,23,26,30,32,33]

The results of collagen and epinephrine-induced platelet aggregation are not consistent.[8,10,18,23,26,29,30,33] Some reports noted a marked inhibition of collagen-induced platelet aggregation. Other investigators did not find these results. The platelet response to epinephrine is also variable. The demonstration of the loss of secondary aggregation without interference of primary aggregation is not consistently seen.

A review of the literature revealed some studies which did not demonstrate in vivo or in vitro changes when aggregation studies were performed.[20,21,31,44] The reasons for these discrepancies may stem from methodology variations in incubation times[4,8,18] and in vivo contact times prior to sampling for platelet aggregation testing.[8] Other discrepancies are in the dosages of antibiotics used in vitro which are clinically unattainable and the concentrations of aggregating agents used in performing the aggregation studies.[8,10,30-32]

Penicillin-induced platelet dysfunction can be detected by prolongation of the bleeding time within 30 minutes to 3 days of starting therapy. Maximum platelet dysfunction in vivo can be identified by performing aggregation studies within 24 hours to 10 days after antibiotic administration, depending upon the dose of antibiotic and the concentration of aggregating agent. The duration of the dysfunction lasts 3-21 days after treatment has been discontinued[4,10,18,23,27,30,32,33] and may be due to an irreversible effect on the platelet.

Episodes of clinical bleeding, such as melena, wound oozing, and hemorrhage are reported with the use of high doses of carbenicillin[3-5,7,9,13,14,19] and penicillin G. Nafcillin was recently associated with

these findings.* Two patients at the University of Utah Hospital who were treated with high doses of nafcillin showed clinical signs of bleeding which resolved upon discontinuation of the antibiotics. Platelet aggregation studies were performed on the first patient and showed marked inhibition of the secondary wave and depression of the primary aggregation response to ADP. After one month without drug administration, the aggregation studies returned to normal. The patient was rechallenged with the same dose of antibiotic and demonstrated recurrence of the effects on the primary and secondary aggregation response.

Nafcillin, a semisynthetic penicillin, is used as a first-line antibiotic for penicillin G resistant staphylococcal infections.[45,46] Only recently was nafcillin-induced platelet dysfunction noted. It has not been previously reported. Therefore, an initial study to investigate this problem was designed.

OBJECTIVES

To evaluate the effect of nafcillin on platelet aggregation.

To investigate the effect of increasing concentrations of nafcillin on the aggregation response.

METHODS

Study Design

This investigation utilized a controlled-paired, equivalent time sample design. Samples were matched for similar platelet count, volume, incubation time and temperature. In addition, the

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* Data on file at the University of Utah Coagulation Laboratory.

aggregating stimuli and duration of the experimental run-times were matched.

The effect of increasing concentrations of nafcillin in PRP was compared to a control in PRP. Three different aggregating agents were used at 3 separate concentrations. The samples were run in duplicate for each concentration of the aggregating agent. (Set one.) The entire investigation was repeated 10 days later to ensure reproducibility. (Set two.)

Preparation of the blood

Blood was collected from 3 fasted, healthy volunteers (2 men, 1 woman) who had not ingested aspirin or any other drugs which could alter normal platelet aggregation for at least 14 days prior to the blood draw. All tubes were sealed with Parafilm® (American Can Co., Greenwich, Conn.) when not in use.

Collection of blood. Blood was drawn in plastic collection tubes and gently mixed immediately with the appropriate anticoagulant (9 parts venous blood and 1 part 0.13 M sodium citrate). All collection equipment and transfer utensils were plastic or siliconized glassware to minimize mechanical activation of the platelets.[47]

Preparation of platelet-rich plasma and platelet-poor plasma (PPP). Fresh, room temperature, citrated whole blood was centrifuged at 205 $\overset{\circ}{g}$ (930 RPM) for 12 minutes. The top layer of PRP was then transferred by plastic pipette to a second plastic tube. The citrated whole blood was respun at 5500 $\overset{\circ}{g}$ (5,000 RPM) for 15 minutes. The plasma layer (PPP) was then transferred to a third plastic tube. The PRP from each volunteer was pooled in equal volume before standardization. The PPP was also pooled before standardization.

Standardization of the platelet-rich plasma. The platelet count of the PRP was determined by the Coulter counter®,[48,49] (Model ZBI, Coulter Electronics, Inc., Hialeah, Fla.). The platelet count in the final plasma was standardized by a mixture of PRP and PPP to obtain a platelet count of 250,000/mm^3. The standardization technique is described in Appendix II.

Preparation of antibiotics

Reconstitution and storage. Sodium nafcillin powder without preservatives (Wyeth Laboratories, West Chester, Penn.) was reconstituted and then diluted with Owrens buffer to final test concentrations. The technique used to prepare Owrens buffer is described in Appendix III. All concentrations of nafcillin were freshly prepared with Owrens buffer and the tubes discarded within 24 hours after reconstitution.

Dilutions of antibiotic. After the antibiotic was reconstituted it was further diluted to final concentrations of 25, 50, 75, 100, 200, and 1000 mcg/ml in PRP (spiked samples*). Dilutions were made according to the procedure outlined in Appendix IV.

Confirmation of test antibiotic concentrations. Similar volumes of antibiotic were added to identical tubes of Owrens buffer to achieve final concentrations of 25, 50, 75, 100, 200, and 1000 mcg/ml. After adequate mixing, a 2 ml aliquot was pipetted into another plastic tube and frozen at -70° C until assayed. These final antibiotic concentrations were assayed microbiologically by the modified method of Bennett et al,[50] using Sarcinia lutae as the test organism.

Preparation of aggregating agents

Aggregating agents were prepared according to the policy and

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* Spiked samples refer to PRP to which nafcillin has been added.

procedures of the Coagulation Laboratory at the University of Utah Hospital. These methods are described in Appendix V. Stock solutions of ADP and collagen were freshly prepared each month and frozen at -70° C. Immediately before use, these preparations were thawed and diluted to give the working solutions of ADP and collagen. Dilutions of epinephrine were freshly prepared from a commercially available product. Standard aggregation response curves of all reagents were recorded. Dilute solutions of ADP, epinephrine, and collagen were discarded after each run.

Adenosine Diphosphate. ADP (Sigma Laboratories, St. Louis, Mo.) was used in final concentrations of 3×10^{-6} M, 1×10^{-5} M, and 1×10^{-4} M.

Epinephrine. Epinephrine (Parke Davis, Detroit, Mi.) was used in final concentrations of 1×10^{-6} M, 1×10^{-5} M, and 1×10^{-4} M.

Collagen. Crude collagen extract (Sigma Laboratories, St. Louis, Mo.) was prepared by a method of Day and Holmsen[52,53] and stored at -70° C until used. Immediately before use, the collagen solution was thawed, rehomogenized and kept at 4° C (ice bath) during the experiment. A standard response curve was prepared by the addition of diluted collagen to PRP until adequate aggregation was demonstrated. Dilutions of this solution were made with 1.67 mM acetic acid to final ratios of 1:8, 1:16, 1:32, and 1:64.

Aggregation studies

Aggregation studies were performed using the instruments and supplies in the Coagulation Laboratory at the University of Utah Hospital.

Method. Aggregation studies were performed by the turbidometric method[52-55] at 37° C using dual aggregometers[51] (Chronolog Corp., Broomall, Penn.). Platelet-poor plasma was used as a standard of the maximum change in optical density. Aggregating agents were added to PRP spiked with nafcillin and control PRP to which Owrens buffer was added. Recordings of the change in optical density were made on a dual channel recorder (Linear Instruments Corp., Irvine, Calif.) outfitted with 10 inch chart paper. All aggregation studies were done in duplicate.

Incubation. Spiked and control samples of PRP were incubated for 1.5 hours at 37° C in a water bath.

DATA ANALYSIS

Calculations

Calculations of the percent response was done by equation #1.

$$\frac{\text{sample optical density change}}{\text{total optical density change}} \times 100 = \% \text{ response} \qquad \text{(eq. 1)}$$

The percent response for both the control and spiked samples were calculated by this method. The percent response (mean ± S.E.M.*) was determined from the duplicate runs.

Hypothesis and data analysis

Null hypothesis: There is no difference in the percent response. If they do not vary significantly, then it is assumed that nafcillin, in the measured concentrations, does not influence platelet aggregation in vitro. Probability (p) values of less than 0.05 were

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* S.E.M. - Standard Error of the Mean

considered significant. Mathematical computations were performed on a Hewlett-Packard^{R} (HP67) programmable calculator (Hewlett Packard Co., Corvallis, Or.). Statistical testing of the hypothesis was performed by analysis of variance (ANOVA).[56]

RESULTS

Collagen

No inhibition of platelet aggregation in control or spiked samples was observed with nafcillin concentrations ranging from 25-200 mcg/ml (Table 1, 2). There was a significant difference between control and spiked samples at 1000 mcg/ml (Table 1). Significant inhibition of the maximum aggregation response was shown with resultant disaggregation of the samples at weak dilutions of collagen (Figure 1). In set one, this effect occurred at a collagen dilution of 1:32 and in set two it occurred at a collagen dilution of 1:64 (Table 2). The difference between the results of the two sets can be explained by the variability in the preparation of the collagen suspensions and not directly to the influence of the drug.

Epinephrine

No significant inhibition of platelet aggregation in the control or spiked samples was observed (Table 3, 4). Weak concentrations of epinephrine (1×10^{-6} M) consistently showed a poor response and in many cases demonstrated a severely depressed secondary wave form in both the spiked and control samples. This effect could not be attributed to the drug because it could be shown in both the control and spiked samples. Duplication of the sample response for each concentration of epinephrine demonstrated a reproducible response (Table 4).

Adenosine Diphosphate

No significant inhibition of platelet aggregation in control or spiked samples was observed at nafcillin concentrations ranging from 25-200 mcg/ml (Table 5, 6). At nafcillin concentrations of 100 mcg/ml and 200 mcg/ml, the aggregation response to weak ADP (3×10^{-6} M) showed a prominent secondary wave form which did not appear in the control samples (Figure 2, 3). Significant inhibition of the secondary wave and depression of the primary response to weak ADP (3×10^{-6} M) was shown in nafcillin spiked PRP at 1000 mcg/ml (Table 7, 8). This effect did not occur in the control samples (Figure 4). Duplication of the sample response for each concentration of ADP demonstrated reproducibility (Table 8).

pH

The pH of the control and spiked PRP samples did not exceed the pH of 8.0 for the duration of the aggregation studies. The pH of the spiked PRP was usually 0.2 pH units below that recorded for the control samples.

Antibiotic concentration

Final antibiotic concentrations were verified for both sets of trials by microbiological assay. The percent recovery of nafcillin-spiked samples ranged from 76-133%.

DISCUSSION

Hemostasis

The human platelet, numbering approximately 200,000 to 400,000 per cubic millimeter, has a life span of 8-10 days. They circulate as smooth, disc-shaped cells that are non-adherent to each other

and normal endothelium.[36,37,47] The interaction of the vessel wall, platelet and coagulation proteins is important in normal hemostasis. The primary goal of these interactions is to arrest bleeding at the site of injury. When a vessel is damaged, the hemostatic mechanisms are initiated with platelet adhesion to the site of injury. After adhesion, aggregation of platelets to each other enlarges the hemostatic plug.

The platelet plug plays an important role in the maintenance of hemostasis. Defective adhesion, aggregation or secretion may result in a poorly formed platelet plug. This may result in a bleeding diathesis associated with clinically evident bleeding, i.e. petechiae, wound oozing and hemorrhage. When a bleeding disorder is suspected, in vitro platelet aggregation studies are useful to determine whether platelet dysfunction is contributing to the bleeding disorder. Platelet aggregation using a number of exogenously added stimuli has advanced our knowledge of platelet physiology and subsequently our understanding of the pathophysiology of many platelet disorders. Aggregating agents which stimulate the platelet by different mechanisms can be used to identify the platelet defect.

Aggregation studies

The results of this study demonstrate that nafcillin results in platelet dysfunction which is similar to that found with other penicillins[3-32] and cephalosporins.[20,28-30,34] Significant inhibition of the aggregation response to weak concentrations of ADP was found with large concentrations of nafcillin (Table 7). This finding is the most consistently reported platelet abnormality in the literature for other penicillins and cephalosporins. The presence of a prominent secondary aggregation was observed in the spiked samples at 100 mcg/ml and

200 mcg/ml of nafcillin with weak ADP (3×10^{-6} M) (Figure 2, 3). This effect did not occur in the control samples. These effects were observed without altering the maximum aggregation response. The presence of the secondary wave usually results from the stimulation of platelets with a weak aggregating agent. This observation was important because nafcillin appears to diminish the effect of ADP on the platelet. The use of weaker concentrations of ADP can show a significant platelet dysfunction at lower antibiotic concentrations.

Abnormal collagen stimulated aggregation was demonstrated at high nafcillin concentrations. This effect is not consistently reported in the literature for penicillins and cephalosporins.[8,10,23,26,29,32] Significant inhibition of the aggregation response to weak dilutions of collagen occurred in PRP spiked with 1000 mcg/ml of nafcillin (Table 1). The collagen dilution producing this effect differed between set one and set two. The reason for this difference is probably due to the method of preparation of the aggregating agent. Significant inhibition in set one was shown at a dilution of 1:32 in the spiked samples. At dilutions of 1:64, neither spiked nor control samples responded. The aggregating stimulus was so weak that neither sample responded. Significant inhibition in set two occurred at a dilution of 1:64. Most of the published literature reports the aggregation of platelets with only one dilution of collagen. The use of this technique probably contributed to the higher antibiotic requirement before the effect was demonstrated.

No conclusion could be made from the results of the epinephrine stimulated platelet aggregation. Many of the results are inconsistent (Table 3, 4). Epinephrine is usually added to PRP last because the

platelet responsiveness improves with time[57,58] and alkaline pH.[59-61] Platelet responsiveness to epinephrine reaches a maximum around 90-120 minutes[58,62] and thereafter declines. The lack of consistent results was probably related to the time factor and decreased platelet responsiveness. In this study the addition of epinephrine did not begin until approximately 2.5 hours after venipuncture. As with collagen, inconsistent findings with epinephrine were found in the literature.[8,10 29-33]

In vitro reproduction of nafcillin-induced platelet dysfunction requires many times the plasma concentrations that can be achieved clinically. This disparity is well recognized with carbenicillin[8-10] and penicillin G.[23,26,29] Therefore, either prolonged contact time with the antibiotic or a metabolite may be involved in this inhibition. Possibly some unrecognized plasma factor may be required.

The role of the incubation period is still unclear. Lederer et al[8] studied the aggregation of PRP to which carbenicillin had been added in vitro. They found that carbenicillin had no immediate effect; but, after two hours of incubation at 37° C, platelet aggregation to ADP (2×10^{-6} M) was inhibited. In contrast, Shattil et al[63] found an immediate effect with carbenicillin and penicillin in PRP when aggregated with ADP (2×10^{-6} M). The reason for this disparity is unclear.

Mechanism

The mechanism of the nafcillin-induced platelet dysfunction is unknown. Platelet adhesion, aggregation and secretion are decreased with penicillin G and carbenicillin. Similar effects on

platelet aggregation were noted with nafcillin.* The results of this study are in agreement with these findings.

Cazenave et al[26] postulated that these antibiotics coat the platelet surface and interfere with membrane receptor activation, although actual binding of penicillin to platelets is not yet demonstrated. Shattil et al[63] found that epinephrine and ADP were competitively inhibited at membrane receptors by large doses of carbenicillin and penicillin. These antibiotics decreased the affinity of epinephrine to α-adrenergic receptors and inhibited the covalent incorporation of an ADP analogue into a platelet membrane polypeptide which may be the ADP receptor.

Penicillins are bound to a variable extent to plasma proteins,[64] bacterial membrane proteins[65],and the membrane proteins of the red blood cells.[66] Padfield et al[67] showed that penicillin G was highly bound to a number of phospholipids in vitro. Ampicillin was shown to be bound much less than penicillin G. Many of these phospholipids are identified in the outer platelet membrane.[40] Since platelet membranes also contain glycoproteins[68] that are irregularly distributed on the surface, further work is needed to determine the penicillin binding characteristics to platelet proteins and phospholipids.

Little is known about the entire sequence of cellular events that follow agonist binding but many critical details of the platelet reaction were elucidated in recent years.[69] After the initial activation of the platelet, it will undergo shape change and centralization of the cytoplasmic organelles. These phenomena are related to the activation of contractile proteins by increased cytoplasmic calcium. These contractile

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* Data on file at the University of Utah Coagulation Laboratory.

proteins resemble actin and myosin which are found in other cells. The processes which activate and control the contractile mechanism are not well defined.

The stimulation of the platelet membrane receptor activates phospholipase enzymes to release arachidonic acid from membrane phospholipids. Arachidonic acid is converted to cyclic endoperoxides which are further converted to thromboxane A_2.[40,70] It is now hypothesized that these products of prostaglandin synthesis may be involved in the iontophoretic transport of calcium from the membrane sites into the cytoplasm.[71] Free calcium in the cytoplasm initiates the activation of the contractile proteins and release of the subcellular organelles. Recently Johnson et al[72] reported that carbenicillin does not affect prostaglandin synthesis. Therefore, the mechanism by which penicillin produces this platelet defect remains unknown. Further investigations are warranted to define this mechanism.

Evaluation

The clinical importance of these effects on platelets is unclear at this time. Clinically evident bleeding was observed in two patients receiving large doses of nafcillin.* Results of platelet aggregation studies performed at that time revealed a nafcillin-induced platelet dysfunction. Similar effects on platelet aggregation were shown in this study. Not all patients who receive large doses of nafcillin will demonstrate clinically evident bleeding but they may show altered platelet function. The hemostatic defect may be accentuated by the patients' underlying disease states or other drugs. The altered platelet

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* Data on file at the University of Utah Coagulation Laboratory.

function may predispose them to bleeding. Further study to investigate the in vivo effects of nafcillin is warranted. Identification of the plasma concentration, time of onset, and duration of the effect is important information to determine the clinical significance of this adverse effect.

CONCLUSION

Platelet dysfunction was shown when large concentrations of nafcillin were incubated with PRP for 1.5 hours when compared to control. Significant inhibition of the aggregation response to weak concentrations of the ADP and collagen were shown in spiked samples at 1000 mcg/ml of nafcillin. Depression of the secondary wave was demonstrated with low concentrations of ADP in spiked samples beginning at 100 mcg/ml of nafcillin. The clinical significance of this study cannot be interpreted at this time. Further study to evaluate these effects on platelets in vivo is warranted.

Table 1 - Raw Data. Collagen-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	Collagen Dilution	Percent of Maximum Aggregation									
		Control		Spiked		p value[a]	Control		Spiked		p value
25 mcg/ml	1:8	83.8	85.0	78.8	82.7	NS[b]	76.8	80.2	80.0	78.8	NS
	1:16	78.0	85.0	81.3	83.8		28.4	21.2	20.0	20.0	
	1:32	75.9	79.3	76.5	75.3		ND[c]	ND	ND	ND	
	1:64	42.5	40.0	35.0	37.5		ND	ND	ND	ND	
50 mcg/ml	1:8	78.8	80.0	85.2	81.3	NS	84.0	78.0	86.3	82.7	NS
	1:16	73.8	75.3	77.5	78.8		80.0	87.5	82.5	81.3	
	1:32	28.8	26.3	25.9	25.0		85.0	81.3	83.8	80.0	
	1:64	ND	ND	ND	ND		57.5	72.5	63.8	66.3	
75 mcg/ml	1:8	82.5	82.5	82.5	82.5	NS	75.6	77.5	80.0	80.0	NS
	1:16	78.8	73.8	80.2	77.8		81.3	78.8	78.8	77.5	
	1:32	68.8	65.0	65.9	65.0		77.5	76.3	77.5	83.8	
	1:64	17.5	20.0	20.0	20.7		72.5	71.3	72.5	70.0	

a Statistical analysis done by analysis of variance, significance $p < 0.05$.
b NS - Not Significant
c ND - Not Determined

Table 1 (Cont) - Raw Data. Collagen-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	Collagen Dilution	Percent of Maximum Aggregation									
		Control		Spiked		p value[a]	Control		Spiked		p value
100 mcg/ml	1:8	77.2	80.0	79.0	70.4	NS[b]	86.3	86.3	83.8	85.0	NS
	1:16	72.5	74.1	77.8	76.3		82.5	82.5	81.3	85.0	
	1:32	22.5	17.5	21.5	21.0		80.0	81.5	83.8	75.3	
	1:64	ND[c]	ND	ND	ND		77.5	76.3	76.3	75.3	
200 mcg/ml	1:8	80.0	81.3	82.7	ND	NS	83.1	79.0	82.5	83.8	NS
	1:16	76.3	80.0	86.3	81.3		77.8	82.7	82.5	82.5	
	1:32	55.0	53.8	55.0	61.3		77.5	80.0	81.3	81.3	
	1:64	20.0	22.5	22.5	22.2		72.5	72.5	77.5	72.5	
1000 mcg/ml	1:8	85.0	82.5	86.3	85.0	<0.01	86.3	85.0	86.3	87.5	<0.01
	1:16	83.8	83.8	77.8	78.0		82.7	80.5	77.5	81.3	
	1:32	80.0	80.0	40.0	42.5		81.3	85.0	80.0	78.8	
	1:64	22.5	25.0	21.3	21.3		83.8	81.3	37.5	41.3	

a Statistical analysis done by analysis of variance, significance $p < 0.05$.
b NS - Not Significant
c ND - Not Determined

Table 2 - Mean Collagen-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	Collagen Dilution	Percent of Maximum Aggregation (Mean[a] ± S.E.M.[b])			
		Control	Spiked	Control	Spiked
25 mcg/ml	1:8	84.4 ± 0.6	80.8 ± 2.0	78.5 ± 1.7	79.4 ± 0.6
	1:16	81.5 ± 3.5	82.6 ± 1.3	24.8 ± 3.6	20.0 ± 0.0
	1:32	77.6 ± 1.7	75.9 ± 0.6	ND[c]	ND
	1:64	41.3 ± 1.3	36.3 ± 1.3	ND	ND
50 mcg/ml	1:8	79.4 ± 0.6	83.3 ± 2.0	81.0 ± 3.0	84.5 ± 1.8
	1:16	74.6 ± 0.8	78.2 ± 0.7	83.8 ± 3.8	81.9 ± 0.6
	1:32	27.6 ± 1.3	25.5 ± 0.5	83.2 ± 1.9	81.9 ± 1.9
	1:64	ND	ND	65.0 ± 7.5	66.6 ± 0.3
75 mcg/ml	1:8	82.5 ± 0.0	82.5 ± 0.0	76.6 ± 1.0	80.0 ± 0.0
	1:16	76.3 ± 2.5	79.0 ± 1.2	80.1 ± 1.3	78.2 ± 0.7
	1:32	66.9 ± 1.9	65.5 ± 0.5	76.9 ± 0.6	80.7 ± 3.2
	1:64	18.8 ± 1.3	20.4 ± 0.4	71.9 ± 0.6	71.3 ± 1.3

a Number of Observations (2)
b Standard Error of the Mean
c ND - Not Determined

Table 2 (Cont) - Mean Collagen-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	Collagen Dilution	Percent of Maximum Aggregation (Mean[a] ± S.E.M.[b])			
		Control	Spiked	Control	Spiked
100 mcg/ml	1:8	78.6 ± 1.4	74.7 ± 4.3	86.3 ± 0.0	84.4 ± 0.6
	1:16	73.3 ± 0.8	77.1 ± 0.8	82.5 ± 0.0	83.2 ± 1.9
	1:32	20.0 ± 2.5	21.3 ± 0.3	80.8 ± 0.8	82.7 ± 1.2
	1:64	ND[c]	ND	76.9 ± 0.6	75.8 ± 0.5
200 mcg/ml	1:8	85.7 ± 4.4	82.7 ± 0.0[d]	81.1 ± 2.1	83.2 ± 0.7
	1:16	78.2 ± 1.9	83.8 ± 2.5	80.3 ± 2.5	82.5 ± 0.0
	1:32	54.4 ± 0.6	58.2 ± 3.2	78.8 ± 1.3	81.3 ± 0.0
	1:64	21.3 ± 1.3	22.4 ± 0.2	72.5 ± 0.0	75.0 ± 2.5
1000 mcg/ml	1:8	83.3 ± 1.3	85.7 ± 0.7	85.7 ± 0.7	86.9 ± 0.6
	1:16	83.8 ± 0.0	77.9 ± 0.1	81.6 ± 1.1	79.4 ± 1.9
	1:32	80.0 ± 0.0	41.3 ± 1.3	83.2 ± 1.9	79.4 ± 0.6
	1:64	23.8 ± 1.3	21.3 ± 0.0	82.6 ± 1.3	39.4 ± 1.9

a Number of Observations (2)
b Standard Error of the Mean
c ND - Not Determined
d Single Value Only

Table 3 - Raw Data. Epinephrine-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	Epinephrine Concentration (M)	Percent of Maximum Aggregation									
		Control		Spiked		p value[a]	Control		Spiked		p value
25 mcg/ml	1×10^{-4}	77.8	76.3	53.8	54.4	NS[b]	78.0	80.0	85.0	80.0	NS
	1×10^{-5}	76.3	76.3	75.3	75.3		86.4	80.5	85.0	80.0	
	1×10^{-6}	26.8	26.3	37.5	35.0		45.0	35.0	52.5	57.5	
50 mcg/ml	1×10^{-4}	55.0	77.5	76.3	80.0	NS	78.8	80.0	77.5	81.3	NS
	1×10^{-5}	76.5	77.5	81.3	81.3		82.8	80.0	78.8	80.0	
	1×10^{-6}	29.0	30.0	30.9	29.6		80.0	75.0	72.5	78.8	
75 mcg/ml	1×10^{-4}	72.5	70.3	61.7	61.9	NS	78.8	76.3	77.5	75.0	NS
	1×10^{-5}	76.3	78.8	75.9	73.8		76.3	73.8	76.3	78.8	
	1×10^{-6}	27.5	24.5	29.3	27.7		72.5	73.8	72.5	73.8	

a Statistical analysis done by analysis of variance, significance $p < 0.05$.
b NS - Not Significant

Table 3 (Cont) - Raw Data. Epinephrine-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	Epinephrine Concentration (M)	Percent of Maximum Aggregation									
		Control		Spiked		p value[a]	Control		Spiked		p value
100 mcg/ml	1×10^{-4}	76.3	76.3	76.3	63.0	NS[b]	83.8	80.0	78.8	ND[c]	NS
	1×10^{-5}	77.5	76.3	73.8	76.3		81.3	80.2	81.3	78.8	
	1×10^{-6}	63.8	47.5	25.0	27.5		37.0	30.0	76.3	77.5	
200 mcg/ml	1×10^{-4}	48.8	53.8	67.9	60.0	NS	78.8	73.4	80.0	78.8	NS
	1×10^{-5}	73.8	65.0	73.8	72.8		77.5	80.0	80.0	81.3	
	1×10^{-6}	32.5	33.8	31.3	31.3		67.5	52.5	73.8	68.8	
1000 mcg/ml	1×10^{-4}	67.5	72.8	30.0	31.3	NS	82.5	81.3	78.8	77.5	NS
	1×10^{-5}	80.2	ND	60.0	51.3		81.3	82.7	83.8	84.0	
	1×10^{-6}	36.3	40.0	31.0	31.0		52.5	38.8	35.4	36.3	

a Statistical analysis done by analysis of variance, signifiance $p < 0.05$.
b NS - Not Significant
c ND - Not Determined

Table 4 - Mean Epinephrine-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	Epinephrine Concentration (M)	Percent of Maximum Aggregation (Mean[a] ± S.E.M.[b])			
		Control	Spiked	Control	Spiked
25 mcg/ml	1×10^{-4}	77.1 ± 0.8	54.1 ± 0.3	79.0 ± 1.0	88.5 ± 2.5
	1×10^{-5}	76.3 ± 0.0	75.9 ± 0.6	83.5 ± 3.0	82.5 ± 2.5
	1×10^{-6}	26.6 ± 0.3	36.3 ± 1.3	40.0 ± 5.0	55.0 ± 2.5
50 mcg/ml	1×10^{-4}	57.6 ± 8.8	75.7 ± 1.9	79.4 ± 0.6	79.4 ± 1.9
	1×10^{-5}	74.9 ± 0.2	76.9 ± 1.9	81.4 ± 1.4	79.4 ± 0.6
	1×10^{-6}	29.6 ± 0.5	30.3 ± 0.7	77.5 ± 2.5	75.7 ± 3.2
75 mcg/ml	1×10^{-4}	71.4 ± 1.4	61.8 ± 0.1	77.6 ± 1.3	76.3 ± 1.3
	1×10^{-5}	77.6 ± 1.3	74.9 ± 1.1	75.1 ± 1.3	77.4 ± 1.3
	1×10^{-6}	26.0 ± 1.5	28.5 ± 0.8	73.2 ± 0.7	73.2 ± 0.7

a Number of Observations (2)
b Standard Error of the Mean

Table 4 (Cont) - Mean Epinephrine-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	Epinephrine Concentration (M)	Percent of Maximum Aggregation (Mean[a] ± S.E.M.[b])			
		Control	Spiked	Control	Spiked
100 mcg/ml	1×10^{-4}	76.3 ± 0.0	69.7 ± 6.7	81.9 ± 1.9	78.8 ± 0.0[c]
	1×10^{-5}	76.9 ± 0.6	75.1 ± 1.3	80.8 ± 0.6	80.1 ± 1.3
	1×10^{-6}	55.7 ± 8.2	26.3 ± 1.3	33.5 ± 3.5	76.9 ± 0.6
200 mcg/ml	1×10^{-4}	51.3 ± 2.5	64.0 ± 4.0	76.1 ± 2.7	79.4 ± 0.6
	1×10^{-5}	69.4 ± 4.4	73.3 ± 0.5	78.8 ± 1.3	80.7 ± 0.7
	1×10^{-6}	33.2 ± 0.7	31.3 ± 0.0	60.0 ± 0.0	71.3 ± 2.5
1000 mcg/ml	1×10^{-4}	70.2 ± 2.7	30.7 ± 0.7	81.9 ± 0.6	78.2 ± 0.7
	1×10^{-5}	80.2 ± 0.0[c]	55.7 ± 4.4	82.0 ± 0.7	83.9 ± 0.1
	1×10^{-6}	38.2 ± 1.9	31.0 ± 0.0	45.7 ± 6.9	35.9 ± 0.5

a Number of Observations (2)
b Standard Error of the Mean
c Single Value Only

Table 5 - Raw Data. Adenosine Diphosphate (ADP)-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	ADP Concentration (M)	Percent of Maximum Aggregation									
		Control		Spiked		p value[a]	Control		Spiked		p value
25 mcg/ml	1×10^{-4}	82.5	81.0	78.8	72.5	NS[b]	88.5	79.5	85.2	88.8	NS
	1×10^{-5}	76.5	77.5	76.3	83.8		78.3	83.8	83.8	85.0	
	3×10^{-6}	59.3	52.5	63.8	58.8		75.0	73.8	59.4	70.4	
50 mcg/ml	1×10^{-4}	83.8	82.5	85.0	82.5	NS	82.5	83.8	82.5	85.0	NS
	1×10^{-5}	76.8	76.5	85.2	81.5		82.5	77.5	78.8	78.8	
	3×10^{-6}	70.4	61.3	70.4	67.1		76.3	80.0	75.0	80.0	
75 mcg/ml	1×10^{-4}	80.0	80.0	77.4	77.4	NS	76.3	80.0	81.3	77.5	NS
	1×10^{-5}	81.3	78.8	75.9	78.6		78.8	76.3	76.3	78.8	
	3×10^{-6}	70.9	63.0	69.0	58.1		77.5	73.8	71.3	73.8	

a Statistical analysis done by analysis of variance, significance $p < 0.05$.
b NS - Not Significant

Table 5 (Cont) - Raw Data. Adenosine Diphosphate (ADP)-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	ADP Concentration (M)	Percent of Maximum Aggregation									
		Control		Spiked		p value[a]	Control		Spiked		p value
100 mcg/ml	1×10^{-4}	76.3	77.5	80.2	80.0	NS[b]	82.5	87.5	80.0	85.0	NS
	1×10^{-5}	75.0	75.0	78.0	79.0		83.1	87.5	85.0	76.3	
	3×10^{-6}	72.5	67.5	66.3	65.0		77.5	76.5	70.0	76.3	
200 mcg/ml	1×10^{-4}	80.0	91.3	82.5	80.0	NS	80.0	76.3	80.0	82.5	NS
	1×10^{-5}	78.8	78.8	82.5	80.0		82.5	80.0	82.5	80.0	
	3×10^{-6}	62.5	70.0	65.0	63.8		80.0	77.5	77.5	78.8	
1000 mcg/ml	1×10^{-4}	See Table 7					See Table 7				
	1×10^{-5}										
	3×10^{-6}										

a Statistical analysis done by analysis of variance, significance $p < 0.05$.
b NS - Not Significant.

Table 6 - Mean Adenosine Diphosphate (ADP)-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	ADP Concentration (M)	Percent of Maximum Aggregation (Mean[a] ± S.E.M.[b])			
		Control	Spiked	Control	Spiked
25 mcg/ml	1×10^{-4}	81.8 ± 0.8	75.7 ± 3.2	84.0 ± 4.5	87.0 ± 1.8
	1×10^{-5}	77.0 ± 0.5	80.1 ± 3.8	81.1 ± 2.8	84.4 ± 0.6
	3×10^{-6}	55.9 ± 3.4	61.3 ± 2.5	74.4 ± 0.6	64.9 ± 5.5
50 mcg/ml	1×10^{-4}	83.2 ± 0.7	83.8 ± 1.3	83.2 ± 0.7	83.8 ± 1.3
	1×10^{-5}	76.7 ± 0.2	83.4 ± 1.9	80.0 ± 2.5	78.8 ± 0.0
	3×10^{-6}	65.9 ± 4.6	68.8 ± 1.7	78.2 ± 1.9	77.5 ± 2.5
75 mcg/ml	1×10^{-4}	80.0 ± 0.0	77.4 ± 0.0	78.2 ± 1.9	79.4 ± 1.9
	1×10^{-5}	80.1 ± 1.3	77.3 ± 1.4	77.6 ± 1.3	77.6 ± 1.3
	3×10^{-6}	67.0 ± 4.0	63.6 ± 5.5	75.7 ± 1.9	72.6 ± 1.3

a Number of Observations (2)
b Standard Error of the Mean

Table 6 (Cont) - Mean Adenosine Diphosphate (ADP)-Induced Platelet Aggregation. Control and Nafcillin-Spiked Samples.

Nafcillin Final Concentration (spiked)	ADP Concentration (M)	Percent of Maximum Aggregation (Mean[a] ± S.E.M.[b])			
		Control	Spiked	Control	Spiked
100 mcg/ml	1×10^{-4}	76.9 ± 0.5	80.1 ± 0.1	85.0 ± 2.5	82.5 ± 2.5
	1×10^{-5}	75.0 ± 0.0	78.5 ± 0.5	85.3 ± 2.2	80.7 ± 4.4
	3×10^{-6}	70.0 ± 2.5	65.7 ± 0.7[c]	77.0 ± 0.5	73.2 ± 3.2[c]
200 mcg/ml	1×10^{-4}	85.7 ± 5.7	81.3 ± 1.3	78.2 ± 1.9	81.3 ± 1.3
	1×10^{-5}	78.8 ± 0.0	81.3 ± 1.3	81.3 ± 1.3	81.3 ± 1.3
	3×10^{-6}	66.3 ± 3.8	64.4 ± 0.6[c]	78.8 ± 1.3	78.2 ± 0.7[c]
1000 mcg/ml	1×10^{-4}				
	1×10^{-5}		See Table 8		
	3×10^{-6}				

a Number of Observations (2)
b Standard Error of the Mean
c Depressed Secondary Wave

Table 7 - Raw Data. Adenosine Diphosphate (ADP)-Induced Platelet Aggregation. Control and Nafcillin-Spiked (1000 mcg/ml) Samples.

ADP Concentration (M)	Percent of Maximum Aggregation													
	Control		Spiked		p value[a]	Control		Spiked		p value	Control		Spiked		p value
1×10^{-4}	83.8	83.8	78.0	80.2	<0.001	82.5	81.3	85.0	86.3	<0.001	75.3	80.0	82.5	ND[b]	<0.001
1×10^{-5}	85.0	82.5	77.4	80.0		82.7	82.5	82.5	78.8		78.3	76.3	73.8	75.3	
3×10^{-6}	77.8	72.5	55.6	49.4		80.0	82.5	48.8	51.3		76.3	73.8	49.4	48.8	

a Statistical analysis done by analysis of variance, significance $p < 0.05$.
b ND - Not Determined.

Table 8 - Mean Adenosine Diphosphate (ADP)-Induced Platelet Aggregation. Control and Nafcillin-Spiked (1000 mcg/ml) Samples.

ADP Concentration (M)	Percent of Maximum Aggregation (Mean[a] ± S.E.M.[b])					
	Control	Spiked	Control	Spiked	Control	Spiked
1×10^{-4}	83.8 ± 0.0	79.1 ± 1.1	81.9 ± 0.6	85.7 ± 0.7	77.7 ± 2.4	82.5 ± 0.0[d]
1×10^{-5}	83.8 ± 0.7	78.7 ± 1.3[c]	82.6 ± 0.1	80.7 ± 1.9[c]	77.3 ± 1.0	74.6 ± 0.8[c]
3×10^{-6}	75.2 ± 2.7	52.5 ± 3.1[c]	81.3 ± 1.3	50.1 ± 1.3[c]	75.1 ± 1.3	49.1 ± 0.3[c]

a Number of Observations (2)
b Standard Error of the Mean
c Depressed Secondary Wave
d Single Value Only

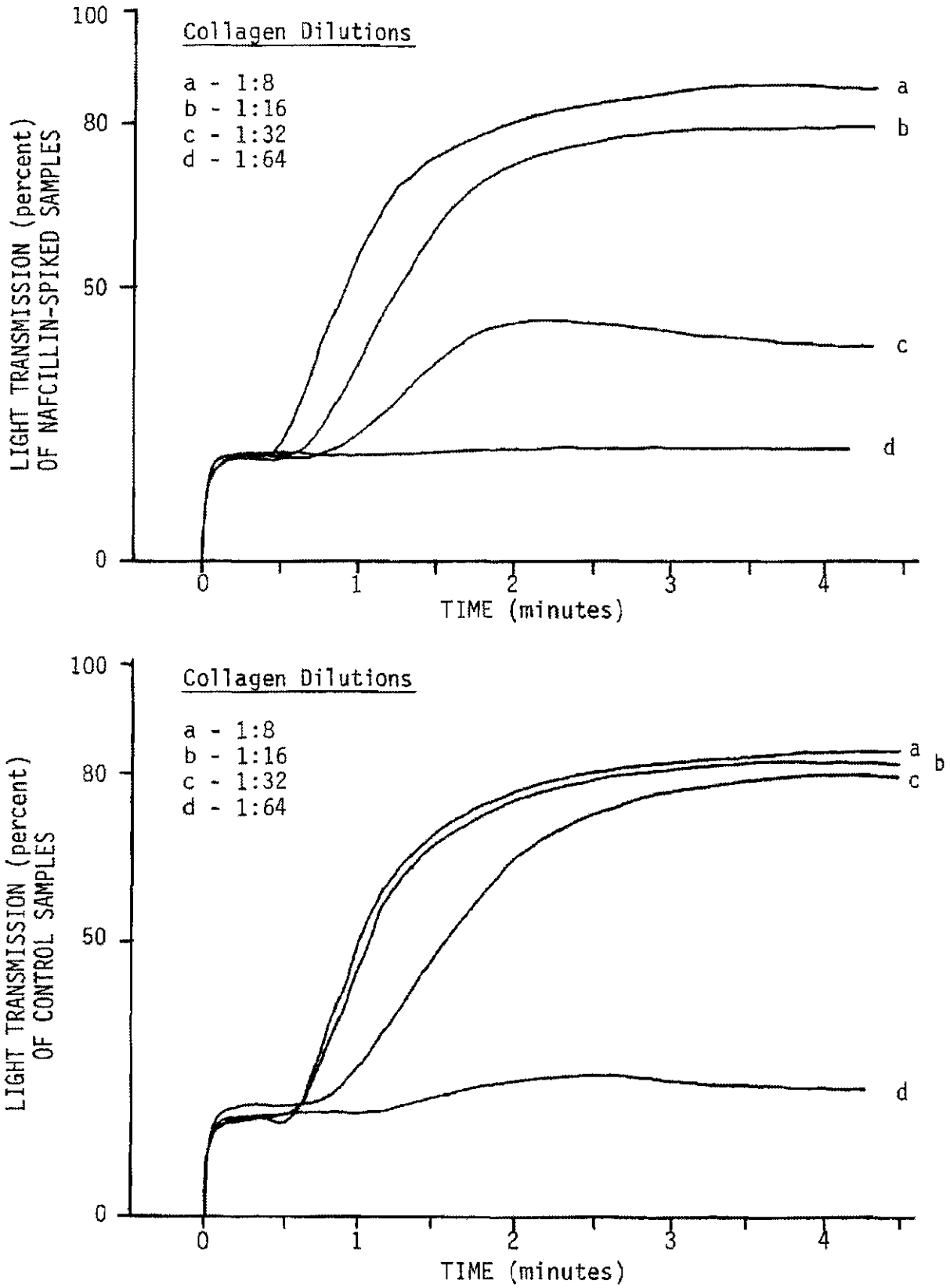

Figure 1 - Platelet Aggregation Response to Collagen. Nafcillin-Spiked (1000 mcg/ml) and Control Samples.

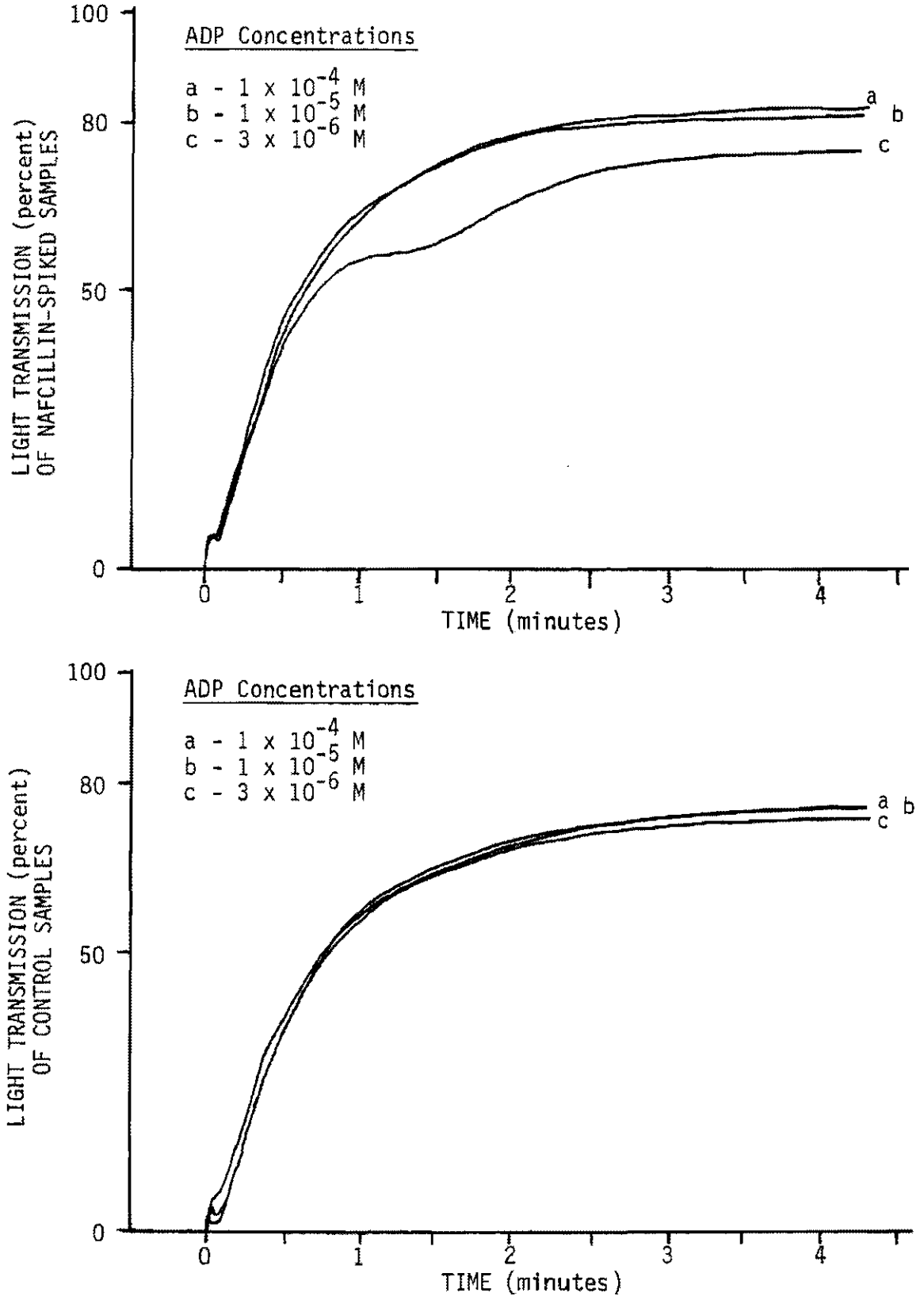

Figure 2 - Platelet Aggregation Response to Adenosine Diphosphate (ADP). Nafcillin-Spiked (100 mcg/ml) and Control Samples.

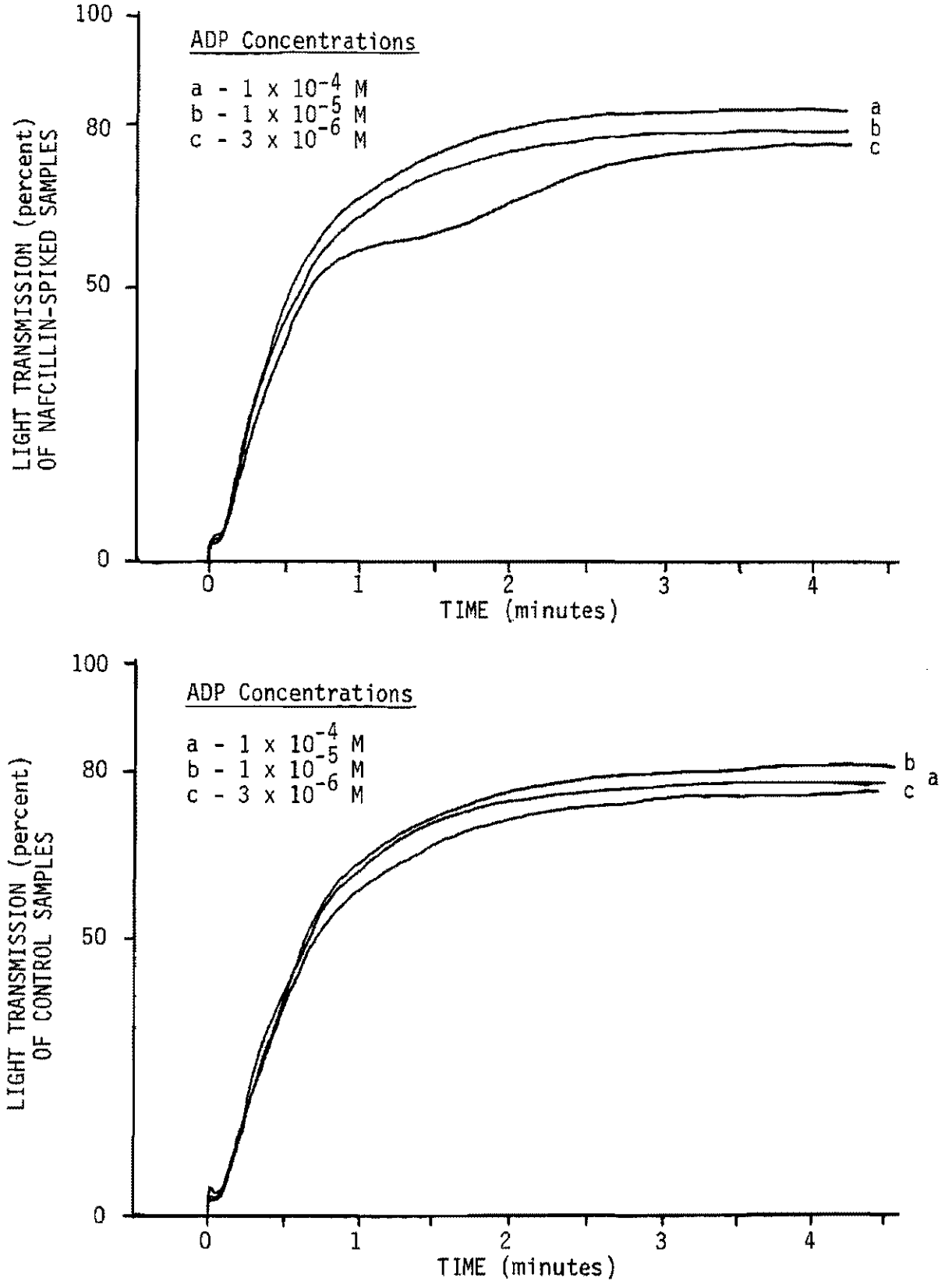

Figure 3 - Platelet Aggregation Response to Adenosine Diphosphate (ADP). Nafcillin-Spiked (200 mcg/ml) and Control Samples.

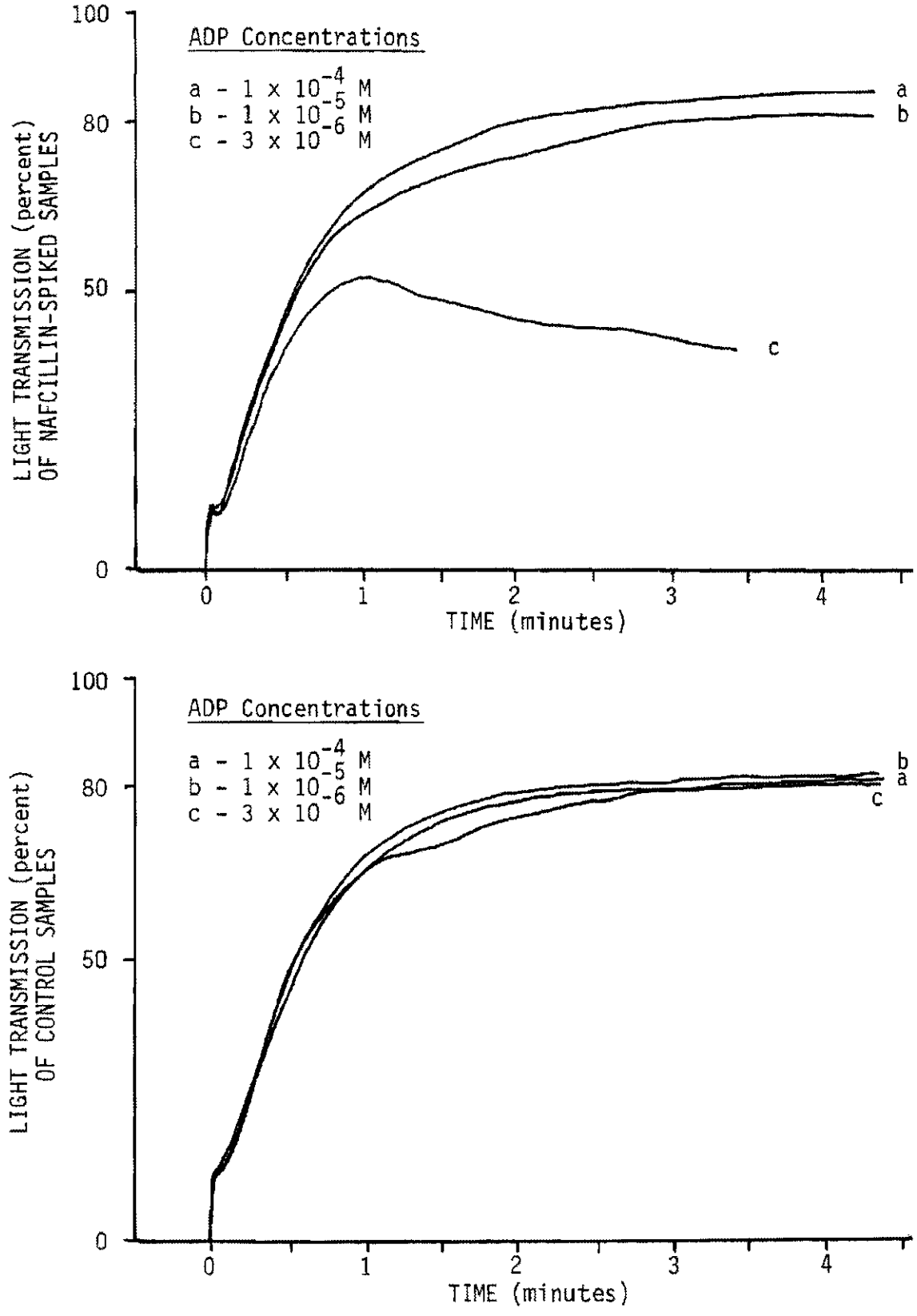

Figure 4 - Platelet Aggregation Response to Adenosine Diphosphate (ADP). Nafcillin-Spiked (1000 mcg/ml) and Control Samples.

APPENDIX I

APPENDIX I

DRUGS THAT IMPAIR NORMAL PLATELET FUNCTION*

Aspirin
Sulindac
Ibuprofen
Fenoprofen
Naproxen
Indomethacin
Tolmetin
Dipyridamole
Sulfinpyrazone
Oxybutazone
Phenylbutazone
Methylprednisolone
Hydrocortisone
Tricyclic Antidepressants
    Amitriptyline
    Nortriptyline
    Imipramine
Propranolol
Cyproheptadine
Dextran
Furosemide
Phentolamine
Chloroquine
Hydroxychloroquine
Clofibrate
Vitamin E
Nitrofurantoin
Penicillin-G
Carbenicillin
Ticarcillin
Ampicillin
Methicillin
Cloxacillin
Cephalothin
Cefazolin

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* Collected from references #1, 2

APPENDIX II

STANDARDIZATION OF PLATELET-RICH PLASMA (PRP)

Platelet concentrations were adjusted to 250,000/mm^3 by diluting PRP with the corresponding platelet-poor plasma (PPP). Platelet count of the PRP was determined by the Coulter Counter®, Model ZBI, then total count correlated by predetermined table[49] provided by the company. Standardization was calculated by equations #1-3.

- $\frac{\text{standard count needed*}}{\text{actual count measured*}} \times$ total volume of standardized PRP required = # ml PRP (eq #1)

  * number of platelets/mm^3

- Total volume of standardized PRP required - # mls PRP = # mls of PPP required for dilution (eq #2)

- Amount of PPP required for dilution - # mls of antibiotic or control solution to be added = actual # mls PPP to be added for standardization (eq #3)

APPENDIX III

PREPARATION OF OWRENS BUFFER SOLUTION

Weigh 5.87 Gm. Sodium di-ethylbarbiturate.
M.W. 207.2

Weight 7.335 Gm. Sodium chloride. M.W. 58.5

Dissolve both compounds in approximately 700 ml of deionized water.

Add approximately 150 ml of 0.1 N Hydrochloric acid.

Titrate to a pH of 7.35 with additional 0.1 N Hydrochloric acid.

Add sufficient deionized water to make 1000 ml total volume.

Store at 4° C.

This solution was made fresh monthly.

APPENDIX IV

DILUTION OF NAFCILLIN

Nafcillin monohydrate sodium (Wyeth Laboratories, West Chester, Pennsylvania), preservative-free powder was used.

Lot # W802416 Expiration Date: August 1983

Concentration, 889 mcg of nafcillin base per 1 mg sodium nafcillin

All dilutions were made with Owrens buffer and adequate mixing. Final concentrations of antibiotic used were 25, 50, 75, 100, 200, and 1000 mcg/ml.

1. Working solution: 25 mcg/ml in final concentration. Weigh* 101.2 mg of nafcillin powder and dilute to 9 ml with Owrens buffer. Then take 1 ml (10,000 mcg) of above mixture and further dilute with 9 ml of Owrens buffer to get a concentration of 1000 mcg/ml.

   Pipette 0.35 ml and add to platelet-rich plasma (PRP) to make final concentration of 25 mcg/ml.

   350 mcg/14 ml = 25 mcg/ml.

2. Working solution: 50 mcg/ml in final concentration. Weigh* 202.2 mg of powder and dilute to 9 ml with Owrens buffer. Then take 1 ml (20,000 mcg) of above mixture and further dilute with 9 ml Owrens buffer to get a concentration of 2000 mcg/ml.

   Pipette 0.35 ml and add to PRP to make a final concentration of 50 mcg/ml.

   700 mcg/14ml = 50 mcg/ml.

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* All weights were measured with a Mettler Balance, Mettler Instrument Corp., Hightstown, N.J., Model H51AR.

3. Working solution: 75 mcg/ml in final concentration.

   Pipette 0.53 ml of solution in #2 (20,000 mcg/ml) and add to PRP to make final concentration of 75 mcg/ml.

   1060 mcg/14 ml - 75.7 mcg/ml.

4. Working solution: 100 mcg/ml in final concentration. Weigh* 202.2 mg of powder and dilute to 9.0 ml with Owrens buffer. Then take 1 ml (20,000 mcg) of above mixture and further dilute with 4 ml Owrens buffer to get a concentration of 4000 mcg/ml.

   Pipette 0.35 ml and add to PRP to make a final concentration of 100 mcg/ml.

   1400 mcg/14 ml = 100 mcg/ml.

5. Working solution: 200 mcg/ml in final concentration.

   Pipette 2 mls of above solution #4 (20,000 mcg/ml) and further dilute with 3 mls Owrens buffer to get a concentration of 8000 mcg/ml.

   Pipette 0.35 ml and add to PRP to make a final concentration of 200 mcg/ml.

   2800 mcg/14 ml = 200 mcg/ml.

6. Working solution: 1000 mcg/ml in final concentration. Weigh* 315 mg of powder and dilute to 10 mls with Owrens buffer to get a concentration of 28,000 mcg/ml.

   Pipette 0.5 ml and add to PRP to make a final concentration of 1000 mcg/ml.

   14,000 mcg/14 ml = 1000 mcg/ml.

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* All weights were measured with a Mettler Balance, Mettler Instrument Corp., Hightstown, N.J., Model H51AR.

APPENDIX V

PREPARATION OF THE AGGREGATING AGENTS

1. Adenosine diphosphate (ADP): (Equine muscle, sodium salt) (Sigma Laboratories, St. Louis, Missouri, Lot #87C-7120).

   a. Stock ADP: 1×10^{-3} M (1 mM) is prepared according to anhydrous weight, purity, and number of water groups attached, which varies with each lot number.

   b. Working ADP Solutions:

      1) Strong ADP - 1×10^{-3} M.

      * 2) Weak ADP - 1×10^{-4} M - dilute 0.1 ml of stock solution with 0.9 ml of Owrens buffer. Mix.

      3) Weaker ADP

      * a) 1×10^{-5} M - dilute 0.1 ml of 1×10^{-4} M solution with 0.9 ml of Owrens buffer. Mix.

      * b) 3×10^{-6} M - dilute 0.1 ml of 1×10^{-5} M solution with 0.23 ml of Owrens buffer. Mix.

* Concentrations to be used.

2. Epinephrine: (Parke Davis, Detroit, Michigan, Lot #7E356).

   a. Stock Solution: 1 mg/ml of a manufactured solution.

   b. Working Solution:

      1) Strong epinephrine - 1×10^{-3} M - dilute 1 ml of stock epinephrine solution with 4.45 ml of deionized water. Mix. Use a tuberculin syringe to measure.

      2) Weak epinephrine - 1×10^{-4} M - dilute 0.1 ml of 1×10^{-3} M with 0.9 ml of deionized water. Mix.

      3) Weaker epinephrine - 1×10^{-5} - dilute with 0.1 ml of 1×10^{-4} M with 0.9 ml of deionized water. Mix.

3. Collagen: (Sigma Laboratories, St. Louis, Missouri, Lot #108C8010)

   a. Stock Collagen: 0.25 gm of dried collagen (bovine achilles tendon) is homogenized in 25 ml of 8.35 mM acetic acid at 4° C in a semimicro-container for a Waring® blender (Waring Products Corp., Winsted, Conn.). Homogenize for 30 seconds at 20,000 rpm at 4° C. Add 25 ml cold distilled water and homogenize for another 30 seconds. Dilute with 200 ml of cold 0.67 mM acetic acid. Store in 2 ml aliquots at -70° C.

   b. Standard Curve: Grind solution until it is almost homogenous. Pour collagen through four layers of 4 x 4 gauze squares into a 17 x 100 mm plastic tube and place on ice. Prepare serial dilutions of collagen using 1.67 mM acetic acid as a diluent. Establish a standard curve; this should be done when the stock collagen is prepared.

   c. Working Collagen: Immediately before use thaw 2-3 tubes of stock collagen and pour into a tissue grinder. Grind solution until it is almost homogenous. Pour collagen through four layers of 4 x 4 gauze squares into a 17 x 100 mm plastic tube and place on ice. Prepare serial dilutions to duplicate those used in establishing the standard curve. (See (b) above.)

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CURRICULUM VITAE

Donald P. Alexander, Jr.

PERSONAL DATA

Home Address: 1222 Medical Plaza
Salt Lake City, Utah 84112
(801)583-6951

Business Address: Department of Pharmacy Practice
University of Utah College of Pharmacy
Salt Lake City, Utah 84112
(801)581-5941

Date of Birth: December 27, 1951

Marital Status: Single

PROFESSIONAL OBJECTIVES

Developing new roles in clinical pharmacy practice, involving the pharmacist in patient care activities, teaching, and clinical research.

EDUCATION

University of Utah College of Pharmacy
Salt Lake City, Utah
Doctor of Pharmacy, Degree expected June 1981

Duke University Medical Center
Durham, North Carolina
ASHP Accredited Hospital Pharmacy Residency Program, July 1978 - June 1979

Ohio State University
College of Pharmacy
Columbus, Ohio
B.S. in Pharmacy, June 1978

PROFESSIONAL EXPERIENCE

Student Clerkships

University of Utah, Department of Pharmacy Practice, Salt Lake City, Utah, July 1979 - present

Responsibilities:

1. Providing clinical pharmacy services
   - medication histories
   - drug information on drug therapy and adverse reactions to medical staff
   - daily work rounds
   - patient drug monitoring
   - pharmacokinetic consults to individualize drug therapy
   - develop alternative treatment plans
   - patient medication counseling
   - in-service education to nursing and medical staff

2. Preceptor for the baccalaureate clinical pharmacy experience

Core Clerkships: Adult internal medicine x 2, surgery, pediatrics, ambulatory pediatric and gerontology clinics, obstetrics and gynecology, psychiatry and drug information.

Elective Clerkships: Adult internal medicine, infectious disease, cardiology, family practice, critical care medicine, and clinical toxicology x 2.

Special Project: Expanded an emergency room-based clinical toxicology consultation service in coordination with the Intermountain Regional Poison Control Center. Responsibilities included initial emergency room assessment with bedside consultation and follow-up management.

Pharmacy Resident, Duke University Medical Center, Durham, North Carolina, July 1978 - June 1979

Responsibilities:

1. Provide clinical pharmacy services
   - daily work rounds
   - patient drug monitoring
   - pharmacokinetic consults on drug therapy
   - patient discharge counseling
   - in-service education

2. Drug distribution services
   - provide drug distribution for 150 beds and supervise a pharmacy technician operated decentralized satellite pharmacy

3. Other activities
   - participation in a 24 hour anticonvulsant consultation service
   - participation in a 24 hour on-call drug information service

Rotation Areas: Public general medicine x 2, cardiology, CCU, hematology-oncology, infectious disease, pediatrics, total parenteral nutrition, ambulatory clinics and drug information.

Pharmacy Intern and Technician, Ohio State University Hospitals, Department of Pharmacy, Columbus, Ohio, July 1973 - June 1978.

Activities: Filling prescriptions with medication counseling to outpatients; proficiency in the use of computerized unit-dose and IV admixture systems; preparation of IV admixtures, hyperalimentation, and sterile unit-dose dosage forms; drug administration to inpatients with experience in emergency code situations; extemporaneous compounding, filling and checking of unit-dose transfer carts.

TEACHING EXPERIENCE

Teaching Assistant, University of Utah, Clinical Toxicology, Fall 1980
Lectures: Salicylate, Acetaminophen
Case Conferences: Coordinate the application of basic principles of toxicology to case presentations
- assessment skills, common poisonings, tricyclic antidepressants, caustics, and petroleum products

Preceptor for the baccalaureate clinical pharmacy experience. This involves a one-on-one contact which introduces the student to the patient care area, interaction with the patient and medical staff and preparation of a drug monitoring system.

Advanced Pharmacotherapeutics Lecture to 1st year Pharm.D. candidates.
Management of Chronic Renal Failure, Spring 1981

In-service Education

Nursing. Aminoglycoside Antibiotic, UUMC, Pediatrics, January 1980
Sedative Hypnotic Agents, Rocky Mountain Gerontology Clinic, May 1980

Medical Staff.
Total Parenteral Nutrition, UUMC, House and attending staff, March 1980
Tricyclic Antidepressants, VA Hospital, House and attending staff, April 1980
Drug Excretion in Breast Milk, UUMC, House staff, June 1980
Antibiotic Use in Otitis Media: An Update, UUMC, House and attending staff, July 1980

Phenothiazine Use in the Elderly, VA Hospital, House and attending staff, August 1980
General Principles in the Management of Poisonings, UUMC, House and attending staff, August 1980

PRESENTATIONS

Peritoneal Transport of Antibiotics. UUMC, Infectious Disease Grand Rounds, October 1980.

Tricyclic Antidepressant Overdose. Primary Children's Hospital, Pediatric Grand Rounds with D. Rollins, M.D., October 1980.

Methods to Enhance Elimination of Poisons From the Body. Continuing Education Conference, University of Utah College of Pharmacy, November 1980.

Salicylate Poisoning. Continuing Education Conference, University Utah College of Pharmacy, November 1980.

Management of a "Tolerant" Staphylococcus aureus Septic Arthritis. Clinical Grand Rounds, 15th Annual ASHP Clinical Midyear Meeting, San Francisco, December 1980. Mary E. Russo and Don Alexander.

MANUSCRIPTS IN PREPARATION

Alexander D, Russo M, Rothstein G, Fohrman D. Nafcillin-induced Platelet Dysfunction."

Russo M, Alexander D. "Antibiotic-tolerant Staphylococcus."

HONORS AND ACTIVITIES--STUDENT

American Association of Colleges of Pharmacy
Council of Students, School Representative, 1975-1978
District Representative, 1977
Regional Representative, 1977

Student American Pharmaceutical Association, 1975-1978

Drug Abuse Committee, Member, 1975-1978
Chairman, 1976-1978

Outstanding Service Award, 1978

Class President, Junior, 1977

Pharmacy Council, Member, 1975-1978

College of Pharmacy Curriculum Committee, 1978

Upjohn Achievement Award, 1978

Kappa Sigma Fraternity, 1969-1978

Rho Chi Honor Society (Pharmaceutical), 1981

PROFESSIONAL ORGANIZATIONS AND LICENSURE

Ohio State Pharmacuetical Association (past)

American Pharmaceutical Association (past)

American Society of Hospital Pharmacists, SIG, Adult Clinical Pharmacy

Central Ohio Society of Hospital Pharmacists (past)

Licensed as a R.Ph. in Ohio (#03-21-2505)

OTHER INTERESTS

Athletics, automobiles, motorcycles, camping, hiking, boating, saling, woodworking and art designs.